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Elimination of the other Opium Alkaloids in the Assay of Opium for Morphine

In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper prepared by the United Nations Secretariat chemists in which the discussion on the assay of opium for morphine is continued.

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ELIMINATION OF THE OTHER OPIUM ALKALOIDS IN THE ASSAY OF OPIUM FOR MORPHINE

Object of the paper

1. The present paper relates to the method for assaying the morphine content of opium as described in document ST/SOA/SER.K/1.

2. In order to prove that no substantial amount of any other alkaloid can be present in the final morphine residue a thorough study has been made of what becomes, during the morphine extraction process, of all other alkaloids known to be present in opium and of several others which might possibly be present. Many of the observations made during the study are applicable also to other methods of assaying opium for morphine.

The Major Alkaloids of Opium

3. The six principal opium alkaloids other than morphine, all of them easily detected in appropriate extractions even of very little opium, are: narcotine, papaverine, codeine, thebaine, cryptopine, and narceine.

4. Narcotine is scarcely present in appreciable amount in the lime-water extract of opium; any portion that may get into the ether-acetone extract would be very readily extracted by chloroform from dichloracetic acid solution.

5. However, one more possibility for narcotine should be considered. If the lactone ring opens, it becomes soluble in alkali, forming a narcotinate. It may then stay with morphine in extraction methods which depend on the solubility of morphine in alkali, and this possibility has sometimes been overlooked. Moreover, any narcotine in the final residue will show some titration, although it is too weak a base to be accurately titrated in the usual way. In order to study this possibility in the United Nations method, narcotine was heated in a solution of alcoholic KOH and thus converted to potassium narcotinate. The alcoholic solution was diluted with water; the narcotine-acid was then set free by addition of ammonium salt, and extracted with chloroform. The residue, upon evaporation of the solvent, was not readily soluble in lime-water, but a little dissolved. After addition of ammonium chloride and continuous extraction with ether-acetone, a small residue of the narcotine-acid was obtained. However, after solution in dichloracetic acid - whether or not this spontaneously reconverted it to narcotine - it was readily extracted by chloroform. The narcotine therefore cannot get into the final morphine extract.

/6. Papaverine

6. Papaverine is present only in very small amounts in the lime-water solution. Like narcotine, it is very readily extracted by chloroform from dichloracetic acid solution. In fact, both narcotine and papaverine can be shaken out gradually from dichloracetic acid solution even by means of carbon tetrachloride when this solvent is used instead of chloroform.

7. Codeine is the second alkaloid to morphine in the lime-water extract, and of course it is extracted by the ether-acetone. It is the most difficult of the opium alkaloids to separate from morphine by the dichloracetic-acid method. However, an excellent separation is made, although it normally requires shaking with from 8 to 14 separate portions of chloroform, and occasionally even more. Codeine could be more readily separated by extraction from alkali, but as the acid separation would still be necessary to separate the phenolic "alkaloid 3", it was decided to use only the acid separation for the regular method of assay.

8. Thebaine is present in the lime-water solution in much greater amounts than might be expected, considering its insolubility as the free base in an alkaline solution. (In one case, in connexion with a codeine determination made on the lime-water extract, thebaine equivalent to 2% of the opium was found.) Thebaine is extracted by chloroform from dichloracetic acid much more readily than codeine though not quite so readily as papaverine.

9. Cryptopine closely accompanies codeine in extractions. It is extracted more readily than codeine by chloroform from dichloracetic acid solution.

10. Narceine is not a phenol, like morphine, but it has a carboxylic acid group which makes it soluble in alkali. In most methods of determining morphine by extraction, narceine accompanies morphine throughout, though it is so feeble as a base that it may not affect the titration. Its isoelectric point is at about pH^6, while that of morphine is about pH^9. In the United Nations method, the adjustment of the pH for the extraction of morphine, and the insolubility of narceine in ether-acetone, are not conducive to its extractions, and practically all of it that is extracted from the opium by the lime-water, remains in the original aqueous solution. Any portion that does go into the ether-acetone extract is very readily shaken out in Stage III. In fact, narceine is more readily extracted by chloroform from dichloracetic acid solution than from an ammoniacal aqueous suspension of the freshly precipitated base.

/The Minor

The Minor Non-Phenolic, Non-Acidic Alkaloids

11. In addition to the preceding alkaloids there are a number of minor alkaloids in opium, both non-phenolic and phenolic. The non-phenolic (and non-acidic) minor alkaloids are: laudanosine, which is closely related to papaverine, but a stronger base; neopine, an isomer of codeine; protopine (or fumarine), closly related to cryptopine; papaveramine, of unknown structure; xanthaline (papaveraldine), an oxidation product of papaverine; and hydrocotarnine, a hydrolysis product of narcotine. The last two are very probably not natural alkaloids of the poppy plant.

12. Neopine is, like codeine, extracted gradually by chloroform from dichloracetic acid solution.

13. Protopine (fumarine) is extracted fairly readily, like cryptopine (to which it is closely related), by chloroform from dichloracetic acid solution.

14. The remaining known minor non-phenolic alkaloids were not available for individual study; but, from their relationships, it is probable that they are all likewise extracted by chloroform from dichloracetic acid solution. At any rate, they would be extracted by chloroform from alkali solution. This separation is not part of the United Nations method, but it has been applied several times after titration of the morphine, and in no case has any important error been discovered. Generally the alkaloid thus recovered has been less than 0.07% of the opium by titration and even this small amount seemed to consist mainly of residual traces of codeine rather than some minor alkaloid. It is therefore certain that the non-phenolic, non-acidic alkaloids of opium, whether major or minor, do not introduce any substantial error into the morphine determination, either individually or in the aggregate.

The Minor Phenolic Alkaloids and Little-Known Bases

15. The minor phenolic alkaloids of opium may be divided into two classes, those extractable by chloroform from alkali solution, and those not so extractable. In the past this distinction was not recognized: it was assumed that with any organic solvent, the minor phenolic alkaloids must stay with morphine when the non-phenolic alkaloids are separated by shaking out an alkali solution; but on the basis of reported percentages for these minor alkaloids it was thought, or at least hoped, that if there were an error, it would be a slight one. Actually most of the minor phenolic alkaloids are separated from morphine when chloroform is used for the

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alkali extraction, but even so the positive error caused by a phenolic alkaloid that stays with the morphine is about 0.3% of the opium, while if some less-effective solvent is used, it may perhaps be twice as much. (These positive errors in extraction methods were, however, often balanced or overbalanced by negative errors.)

16. In extractive studies made at the beginning of the present research, the little-known and obscure alkaloids of opium that could be recognized in some way were first distinguished by number as follows: alkaloid 1, the alkaloid that yields a purplish red colour on heating in solution with mineral acid ("porphyroxine-meconidine"); alkaloid 2, the chief phenolic alkaloid extractable by chloroform from alkali solution (laudanine); alkaloid 3, the chief phenolic alkaloid (after morphine) not extractable by chloroform from alkali solution; alkaloid 4, insufficiently studied, but meant to be the chief "alkaloidal" substance not readily extracted from aqueous solution by immiscible organic solvents, but extracted to some extent by the combinations of solvents such as are used to extract morphine, by reason of some solubility in the water-miscible component of the solvent mixture (e.g. isopropanol). These four probably account for all the phenolic or acidic bases, after morphine and narceine, that may amount to or possibly exceed 0.1% of the opium. In extractive separations on a larger scale the following have also been distinguished, all apparently of phenolic character: alkaloid 5 which gives a colour reaction like that of thebaine with sulphuric acid; alkaloid 6, a very weak base and extractable by chloroform from alkali solution; alkaloid 7, which closely accompanies alkaloid 2 (and like it, is extractable by chloroform from alkali solution), but which yields colour reactions like those of alkaloid 3.

17. Alkaloid 1 --- referring to the true alkaloid which has been erroneously described under the names "porphyroxine" and "meconidine" --- is apparently phenolic, or at any rate, soluble in alkali, but is extractable by chloroform from alkali solution, and also from dichloracetic acid solution. Contrary to statements in the literature, there is no "porphyroxine" that is insoluble in alkali, nor is there any "meconidine" that is not extractable by chloroform from alkali. Rhoeadine, another alkaloid which yields a red colour by heating its solution in dilute mineral acid, is not an alkaloid of opium though often so listed.

18. Laudanine has been found, contrary to previous statements, to be readily extracted from alkali solution (2% NaOH) by chloroform. It is also readily

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extracted by chloroform from dichloracetic acid solution. This was ascertained both on known laudanine and on the "alkaloid 2". The known laudanine was obtained from the Späth collection, through the kindness of Professor Fuchs, Head of the Pharmacognostical Institute of the University of Vienna, Austria. Laudanidine (tritopine) is an optical isomer of laudanine and is doubtless included in the extract of "alkaloid 2" from opium.

19. "Alkaloid 3" is not extractable from alkali solution by chloroform, and accompanies morphine in most extractions. It is, however, separated from morphine by extraction with chloroform from dichloracetic acid solution, six or seven shakeouts being required. It can also be separated from morphine by the far greater solubility of the free base in carbon tetrachloride; but this is difficult to apply to extractions unless the quantities are so small that the morphine does not precipitate during the shakeout with carbon tetrachloride; and the separation is also less complete than with chloroform and dichloracetic acid solution, unless the process is repeated once or twice. It was chiefly to separate this alkaloid that the dichloracetic acid separation was worked out. Probably either this base or "alkaloid 7" is to be identified with codamine. Codamine is said not to be extracted from alkali by chloroform and on this basis it should be "alkaloid 3"; but it seems probable that the same mistake was made as for "meconidine" and laudanine, and that it is "alkaloid 7" that is really codamine.

20. Other solvent-combinations used for morphine take out more extraneous material from opium solutions than does 60:40 ether-acetone. If a continuous extraction is made with ether-acetone as in the United Nations method, until the morphine is all out, and then benzene-isopropanol (3:1) is used, a two or three hour extraction brings over substances amounting to some 2 or 3 per cent of the opium and yielding precipitates with the "general alkaloidal reagents". A substantial part of this material is extracted by chloroform from dichloracetic acid solution, including a substance giving a strong blue or green colour with Fröhdés reagent. The small amount of the remaining material that may be re-extracted by the morphine solvents apparently does not yield any titration, as long as the residue does not contain any traces of residual morphine or "damaged" morphine. (See below, paragraphs 28 to 32). Thus, most of the "alkaloid 4" material is excluded anyway by the ether-acetone extraction, but it does not appear that any error is introduced even if the first extraction is made with benzene-isopropanol. The ether-acetone extraction

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may be completed more quickly by the use of a much larger proportion of acetone than was originally specified. Although more extraneous material comes over than with 60:40 ether-acetone, it is less than with benzene-isopropanol, and this change in the method seems to be for the better.

21. "Alkaloid 5", which gives a thebaine-like colour reaction with (2 + 1) sulphuric acid, is apparently a phenol and perhaps a diphenol. It has scarcely been found in more than traces in ether or ether-acetone extracts from the lime-water solutions. Most extractions of it are incomplete, and it is associated with the strong development of yellow or brown colours in alkaline solution. It is extracted more readily by chloroform from alkali solution than by chloroform from dichloracetic acid solution. However, if the colour development is indeed due to this alkaloid, it is much more harmful to the morphine determination on that account than by reason of the negligible amount of base that might get into the final morphine residue.

22. "Alkaloid 6" is perhaps to be identified with lanthopine. It is a very weak base which is readily extracted by chloroform from dichloracetic acid solution, and is also extractable by chloroform from alkali solution.

23. Either "alkaloid 7" or "alkaloid 3" is presumably codamine; although the green colour given by nitric acid -- which is supposed to identify codamine -- is very transient, in fact a glaucine-type reaction, as actually observed with residues of "alkaloid 7" or "alkaloid 3". "Alkaloid 7" closely accompanies "alkaloid 2" (laudanine), and in spite of discrepancies in the description it probably is codamine, which is an isomer of laudanine. At any rate "alkaloid 7" is extractable by chloroform either from dichloracetic acid solution or from alkali solution.

Other Possible Alkaloids

24. Thebenine is a phenolic base which does not occur naturally, but is formed from thebaine when the latter is boiled in a strongly acid solution (e.g., 10 per cent HCl). Treatment with hot acid solution, sufficiently drastic to convert the thebaine, has been used in some proposed methods of opium assay. Thebenine is not extracted from alkaline solution by chloroform, but it is extracted by chloroform from dichloracetic acid solution. After the latter shakeout in an experiment, ammonifying the aqueous solution and extracting with chloroform-isolutanol yielded

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only a slight residue with no titration. Thus, even if thebenine were present at the start, it does not appear that it could produce any error in the morphine titration by the United Nations method. Due to the formation of coloured products from the readily oxidizable thebenine it is inadvisable to use any method of extracting the opium which will convert the thebaine. Of course, the extraction of the opium with lime-water, as in the present United Nations method, does not produce any thebenine.

25. Orthodiphenol-oxymorphine - This name is used here for the oxidation product of morphine which is an orthodiphenol; it is formed in various oxidations of morphine instead of pseudomorphine. Some of this substance was tried in the morphine method. After addition of ammonium chloride to the lime solution, ether-acetone extracted more than half the quantity (about 26 mg. out of an original 40 mg.) in a few hours. It was not extracted readily by chloroform from dichloracetic acid; 10 shakeouts extracted only about 8 mg. If present in any substantial amount, some would probably get into the final morphine residue. However, after taking care to remove residual traces of morphine, an attempt to titrate about 13 mg. showed no titration value whatever. This substance has never been identified in opium, nor has any evidence of it been found in the morphine determinations made by the United Nations Secretariat.

26. Pseudomorphine, an oxidation product of morphine, is almost certainly not a natural alkaloid of the poppy plant. In the factory separations by which it has been obtained from opium it was probably formed by the chemical processing; but the possibility of small amounts being present in some opiums can hardly be excluded. Pseudomorphine was tested in the United Nations method under discussion. It was soluble in lime-water, and though it was thrown out of solution when ammonium chloride was added, very little (only some 3 mg. out of 40 mg.) was carried over by ether-acetone, in several hours extraction. Pseudomorphine, like morphine, is not extracted by chloroform from dichloracetic acid solution. The base is not readily soluble in organic solvents, but with the conditions the same as for morphine, more or less is extracted (with chloroform-isobutanol 21 mg. were recovered out of 50 mg.). Furthermore, although a much weaker base than morphine, it does yield a titration value. Since pseudomorphine is probably not substantially present in opium, and is scarcely extracted by ether-acetone, about

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the only way it could be present in the residue dissolved by dichloracetic acid would be as a result of formation from morphine in the ether-acetone receiving flask, or in the course of evaporation of the solvent solution. In such circumstances a little would get into the final morphine residue, but the net effect would be negative, due to destruction of morphine. Any morphine converted into pseudomorphine in the original lime-water solution would almost all be lost. Thus, if any pseudomorphine should be found in the course of the determination, it would most probably indicate a real loss of morphine, rather than an apparent gain. No indication of pseudomorphine has been noted in the extractions made by the Secretariat, but some damage to the morphine molecule, not resulting in recognizable pseudomorphine, does occur (see below, paragraphs 28 to 32).

27. Neomorphine is an isomer of morphine, having the same relation to it that neopine does to codeine. It has never been shown to occur in opium, but since neopine seems to be a natural opium alkaloid, it is reasonably probable that a little neomorphine occurs naturally, too. Some known neomorphine was kindly furnished to the United Nations Secretariat for experiments by Dr. Lyndon F. Small, National Institutes of Health, Bethesda, Maryland, U.S.A. Although neomorphine is very much more soluble in water than morphine, it is extractable under the same conditions. During extraction, however, and while the free base is in solution, the molecule is very easily damaged, presumably by oxidation, so that identifying colour and crystal tests are injured or even destroyed. Some doubtful residues obtained from opium extracts were studied, and were concluded to be damaged morphine, not damaged neomorphine. No definite evidence for the presence of neomorphine in opium was found in these studies, and it seems unlikely that there can be any significant error in the morphine titration because of the presence of neomorphine. However, the scientific question of its possible presence in opium in very small proportion is still an open one.

28. Damaged Morphine - When ether containing peroxides was inadvertently used for several ether-acetone extractions, it became extremely difficult to extract the damaged morphine from ammoniacal solution in the next-to-last stage of the method; so difficult, in fact, that it was almost impossible to complete the extractions. Moreover, the crystal tests for morphine in the late extracts were spoiled by the damage to the molecule. The colour tests were less affected; at most the response to Marquis' reagent was slightly delayed, the colour with Fröhde's

/reagent

reagent more blue, and the Mecke's reaction much weaker. The damaged morphine still yielded a titration. The damage to the morphine molecule was presumably due to oxidation, but it was not found possible to detect pseudomorphine.

29. It is imperative that pure ether be used for alkaloidal extractions. The ether should be tested by shaking a small portion with some acidified KI solution. If a brownish-yellow colour appears promptly, the ether needs to be purified, which can be done by shaking with $NaHSO_3$ solution and then washing with water. Numerous warnings have been previously given that the purity of ether used for alkaloidal extractions must be verified or an anti-oxidant routinely used. The article by Charles E. Morgan, chemist of the New York Racing Commission, "Extraction of Minute Amounts of Morphine", (Industrial and Engineering Chemistry, Analytical Edition, 9, 383-384 (1937)), although relating only to micro quantities of morphine, may be particularly mentioned. Mr. Morgan has informed the United Nations Secretariat chemists that sodium sulphite solution has been found a more useful antioxidant than pyrogallol.

30. When pure ether is used in the United Nations method, there is usually not enough damaged morphine to cause any concern. However, with certain samples some difficulty in completing the final extraction is noted, particularly if tests are made with Marquis' and Fröhdé's reagents. (See the discussion of Stage IV of the original method, paragraph 35 of ST/SOA/SER.K/1). In such cases it is possible that the total amount of damaged morphine, though very small in comparison with the amount formed by impure ether, may still be over 0.1 per cent of the opium, and the question arises whether it is all formed in the course of the ether-acetone extraction, or whether some damaged morphine may be present in the opium, before analysis is begun.

31. When some of the morphine is damaged during the analytical process (but not so much as to make subsequent extraction impossible), the effect is only to lower the final titration value, due to difficulty in completing the final extraction. On the other hand, if some damaged morphine already exists in the opium before analysis, it ought not to be included (even partially) in the assay; but with normal raw opium such a positive error must be quite small, and perhaps not even appreciable.

/32. Further

32. Further experiments will be made on methods of preventing any damage to the morphine molecule during the analytical process. These will include: (1) Use of antioxidants; (2) Shortening the time during which the morphine is present as the free base, or undergoing extraction, or in alkaline solution; (3) Substitution or use for comparison purposes of other solvent combinations not containing such a sensitive solvent as ether, e.g., benzene-acetone or benzene-isopropanol. When everything feasible has been done to ensure that no damage is done to the morphine during the analytical process, it will be possible to decide whether any "damaged morphine" already exists in some types of opium before analysis.

Conclusion

33. The principle on which the United Nations method for assaying the morphine content of opium is based is the thoroughly sound one of isolating, weighing, and titrating all the morphine and nothing but morphine from a given quantity of opium or a suitable aliquot of the lime-water extract. In this paper the elimination of the other naturally occurring alkaloids of opium -- both major and minor, non-phenolic and phenolic -- has been accounted for, to the limits of our present knowledge and availability of the known alkaloids. In a subsequent paper, various possible means for improving the United Nations method will be presented.